

Antibiotic susceptibility and phylogenetic analyses for the origins and serotypes of *Listeria monocytogenes* strains isolated from ice cream and cream cakes

Orkun BABACAN* 

Department of Veterinary, Programme of Laboratory Assistant and Veterinary Health,
Balıkesir University Kepsut Vocational School, Balıkesir, Turkey

Received: 26.03.2019 • Accepted/Published Online: 18.07.2020 • Final Version: 27.10.2020

Abstract: *Listeria monocytogenes* is a zoonotic bacterium which also infects humans. The aim of this study was to isolate this organism from cream cakes and ice cream and obtain 16S rRNA and hlyA gene sequences from isolates in order to perform phylogenetic analyses. Serotypes and antibiotic susceptibility were also determined. The cream cake and ice cream samples were examined for *L. monocytogenes* according to ISO 11290-1 and using the mini Vidas LMO 2 kit procedure. Antibiotic susceptibilities were investigated using the disc diffusion method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) standards. The Sanger DNA sequencing method for phylogenetic analysis was used for *L. monocytogenes* isolates. A total of 16 (n =128, 12.50%) *L. monocytogenes* strains, 9 (12.16%) from cream cake samples and 7 (12.96%) from ice cream samples, were isolated. This was the first investigation involving sequencing of *L. monocytogenes* isolated from cream cakes and ice creams in Turkey. All isolates were susceptible to sulfamethoxazole/trimethoprim, tetracycline, streptomycin, gentamicin, meropenem, and erythromycin. The numbers of isolates resistant to sulbactam/ampicillin, penicillin G, chloramphenicol, and ampicillin were 16, 2, 1, and 1, respectively. Moreover, 3 isolates showed intermediate resistance to amikacin and 2 to ciprofloxacin. The hlyA gene sequences of 11 of the *L. monocytogenes* isolates isolated from milk were closely related to the hlyA gene sequences of the GenBank reference strains. The comparison of the 16s rRNA gene sequences of the *L. monocytogenes* strains with the GenBank reference serotypes identified 1 isolate as serotype 1/2c, 1 as serotype 1/2a, and 1 as serotype 4. Nucleic acid sequencing is useful for identification of *L. monocytogenes*. The 16S rRNA and hlyA sequences can be used to determine the origin and relationship between *L. monocytogenes* isolates, as well as the serotype.

Key words: Antibiotic resistance, cream cake, ice cream, *Listeria monocytogenes*, phylogenetic, sequence

1. Introduction

Listeria monocytogenes is widespread in the environment, and it is an opportunistic pathogen for both humans and animals. It is a facultative anaerobic Gram positive, rod-shaped bacterium which grows at temperatures between 1.5 and 45° C. *L. monocytogenes* is an intracellular pathogen, multiplying inside the cells of its host [1–3].

L. monocytogenes causes meningitis, septicaemia, rhombencephalitis, and premature birth and abortion in humans and animals [3]. Newborn infants, elderly people, immunosuppressed individuals, and pregnant women and their foetuses are considered as the main risk groups in human infections [1]. The infection is usually transmitted to humans via foods—particularly milk, milk products, and ready-to-eat foods [1,4–6]. The contamination of raw milk is generally related to farm hygiene and management including the milking process, milking equipment, and workers. *L. monocytogenes* is one cause of mastitis in cows.

Poor hygiene during milking as well as during storage and transport is among contamination sources of raw milk by *Listeria* spp. Milk can be directly contaminated by *L. monocytogenes* because of mastitis, encephalitis, or abortion in cattle. Contamination with *L. monocytogenes* can also occur via the processing environment and equipment during manufacture of milk products and other foods. *L. monocytogenes* may produce biofilm. Therefore, it may appear in food processing plants as part of biofilm [7]. For this reason, and in order to eliminate other pathogens such as *Salmonella*, heat treatment is used during the manufacture of ice cream. Ice cream generally has a near neutral pH, allowing *L. monocytogenes* and many other microbes to grow [8]. However, in normal conditions, ice cream is stored and sold at temperatures below –12 °C, which prevents multiplication of all microbes, including *L. monocytogenes*. However, *Listeria* survives very well in frozen ice cream [7,8]. It is important to note that,

* Correspondence: orkun_babacan@hotmail.com

although *L. monocytogenes* eliminated by pasteurisation, post-pasteurisation contamination with low numbers of *Listeria* in the final ready-to-eat product can result in the presence of high numbers after subsequent chilled storage [6]. For this reason, heat treatment and hygiene standards must be carefully applied [9]. Cream cakes provide a good nutrient medium for *L. monocytogenes* to maintain its growth and vitality when the growth characteristics of the bacteria are taken into account. As cream cakes are stored at chill temperature and are ready-to-eat, they pose a risk for *L. monocytogenes* infection in terms of public health [3,10].

Besides the ISO 11290 standard for the detection and identification of *L. monocytogenes* from food and animal feeding materials [11,12], there are also genotyping methods and automated systems which are approved and certified by various authorities. One of these automated systems is VIDAS (Biomerieux, France). VIDAS detects food pathogens including *L. monocytogenes* using enzyme-linked fluorescent immunoassay methods with specific kits such as LMO 2 [11]. Also, the VITEK 2 system (Biomerieux, France) is an automated identification system for bacteria and for antibiotic susceptibility tests using card systems. For the identification of *L. monocytogenes* from suspicious colonies, a Gram-positive identification card is used in the VITEK 2 system. This system reads the cards every 15 min by performing kinetic analysis. The system combines multichannel fluorimeter and photometer readings to record fluorescence, turbidity, and colorimetric signals [12]. In bacterial taxonomy, nucleic acid sequencing and sequence analysis have become quite popular in recent years [13,14]. Today, the 16S rRNA gene sequence is used as the main source of phylogenetic data for the identification of bacteria and the detection of differences between strains or species. A number of studies have used 16S rRNA in the identification, differentiation, and comparison of *L. monocytogenes* from various sources [14–16].

Most human infections with *L. monocytogenes* are sporadic and not attributed to any source. However, according to the European Food Safety Authority (EFSA), 4 out of 37 outbreaks of listeriosis were attributed to milk and milk products, all of which were cheese, between 2008 and 2015 [17]. An outbreak in the US in 2010–2014 was attributed to ice cream [7]. Also, 186 (27%) of 690 reports under the RASFF (Rapid Alert System for Food and Feed) between 2008 and 2016 concerned *L. monocytogenes* in milk and milk products, and 1 (0.1%) was attributed to ice cream and desserts [17]. The US Centres for Disease Control and Prevention (CDC) declared that in 2017 2 of 8 deaths from listeriosis were caused by cheese produced from raw milk [10].

L. monocytogenes is generally susceptible to the following antibiotics: penicillin, ampicillin, erythromycin,

tetracycline, and trimethoprim/sulfamethoxazole [3]. Today, penicillin or ampicillin combined with gentamicin is used as the first option for treating listeriosis in humans. Trimethoprim/sulfamethoxazole, vancomycin, or erythromycin are used as alternative options for treating pregnant women [2]. In veterinary medicine, similar antibiotics are used to treat mastitis caused by *L. monocytogenes* [18].

In Turkey, microbiological controls on foods are performed based on the regulations in the Turkish Food Codex Microbiological Criteria, which state that *L. monocytogenes* should be absent from 25 g of ice cream and cream cakes [19]. Most investigations into *L. monocytogenes* and its antibiotic susceptibility in Turkey have been conducted on raw and pasteurised milk, butter, and cheese as well as meat [2,20–24]. There are few studies about *L. monocytogenes* in cream cakes and ice cream [24].

In this study, we aimed to determine 16S rRNA and *hlyA* gene sequences of *L. monocytogenes* strains isolated from cream cakes and ice cream in order to perform phylogenetic analysis to determine the source of infection and serotypes, and also to investigate antibiotic susceptibilities.

2. Materials and methods

In the study, 74 cream cake samples and 54 ice cream samples offered for sale in different stores (markets, patisserie, etc.) were examined for the presence of *L. monocytogenes* in Giresun province, Turkey between 2013 and January 2017. Cream cake and ice cream samples were selected randomly, and they were bought in their original packages and/or as sold to consumer. While cream cakes and prepacked ice cream samples were taken in their original packaging, approximately 300 g was taken from unpackaged ready-to-eat traditional handmade ice cream placed in a container supplied by the shop. The samples were transported to the laboratory under aseptic and cold chain conditions (+2–+8°C) in a portable refrigerator and examined within half an hour.

2.1. Isolation and identification

The cream cake and ice cream samples were examined simultaneously for *L. monocytogenes* according to ISO 11290-1 [25] and the mini Vidas LMO 2 kit procedure [26] as described below.

The first and second steps were the same for both methods (as specified in ISO 11290-1). For this, 25 g of each sample was weighed into sterile sample bags and 225 ml Half-Fraser Broth (Biomerieux, France) were added before homogenisation in a stomacher for 1 min (Smasher, Biomerieux, France). Homogenised samples were incubated at 30 °C for 24±2 h for preenrichment. Then, 0.1 ml was inoculated into 10 ml Fraser Broth (Biomerieux, France) for enrichment and incubated at 37 °C for 24 and 48 h.

The third stage was different in the mini Vidas LMO 2 kit procedure. After 24 h, 500 μ l of the Fraser Broth enrichment culture was transferred to the LMO 2 kit (Biomérieux, France) and the kit was placed in the mini Vidas device. If the samples were positive for *L. monocytogenes* in the Vidas, the ISO 11290-1 method was continued, using Fraser Broth after 48 h incubation. This involved streaking the enrichment culture onto 3 different selective agar media—Ottoviani and Agosti (Merck, Germany), PALCAM (Oxoid, UK), and Rapid' *L. mono* (Bio-Rad, USA)—and incubating at 37 °C for 24–48 h.

Presumptive *L. monocytogenes* colonies were picked and purified by streaking onto Ottoviani and Agosti (Merck, Germany), PALCAM (Oxoid, UK), and Rapid' *L. mono* (Bio-Rad, USA) agars before being resuspended in tubes containing 3 ml sterile saline to McFarland turbidity 0.5–0.63, and identified using Gram positive identification cards in the Vitek 2 (Biomérieux, France) device. The isolates were then verified as *L. monocytogenes* using the CAMP test with *Staphylococcus aureus* (ATCC® 25923™). Confirmed cultures of *L. monocytogenes* were stored on beads (Cryobank, Mast) at –20 °C. *L. monocytogenes* (ATCC® 19111™) was used as control during isolation, identification, and PCR tests.

2.2. Antibiotic susceptibility of *L. monocytogenes* isolates

Antibiotic susceptibilities were investigated based on the disc diffusion method according to the standards of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) [27–30]. To this end, the isolates were taken from the beads and inoculated in Brian Heart Infusion (BHI) Broth (Biomérieux, France) and incubated at 37 °C for 24 h, followed by streaking onto the 3 isolation media listed above, and incubated at 37 °C for 48 h. The cultures were harvested into sterile saline and adjusted to McFarland 0.5 turbidity. Each suspension was surface inoculated onto Mueller-Hinton F agar (Merck, Germany) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) procedure. Incubation was at 35 \pm 1 °C.

The inhibition zones measured for ampicillin, erythromycin, trimethoprim-sulfamethoxazole, and meropenem were recorded as suggested by EUCAST for *L. monocytogenes*. The zones for the other antibiotics (gentamicin, streptomycin, chloramphenicol, ciprofloxacin, amikacin, penicillin G, sulbactam/ampicillin, and tetracycline) were interpreted according to CLSI (2012 and 2014) for other bacteria [28–31]. *Staphylococcus aureus* (ATCC® 25923™) and *Escherichia coli* (ATCC® 25922™) were used as references.

2.3. Phylogenetic analysis of *L. monocytogenes* isolates

The isolates were incubated overnight in BHI broth (Biomérieux, France) at 37 °C by shaking at 200 rpm

prior to the extraction and purification of DNA in a GeneMATRIX Tissue & Bacterial DNA Purification Kit containing DNA binding spin-columns (EURX, Poland).

Commercial oligonucleotide primers were used for the amplification of the *16S rRNA* and *hlyA* genes in PCR as previously described (14). The amplification process for the DNA of *16S rRNA* and *hlyA* genes was performed as previously described (14), through universal and *hlyA* primers, 27F (5'-AGAGTTTGATCMTGGCTCAG-3'), 1492R (5'-GGYTACCTTGTTACGACTT-3'), *hlyA*-F (5'-GCAGTTGCAAGCGCTTGGAGTGAA-3') and *hlyA*-R (5'-GCAACGTATCCTCC AGAGTGATCG-3'), respectively [14]. Amplifications were performed via a thermal cycler (Turbocycler 2, Blue-ray) under the following conditions: for the *16srRNA* gene: initial denaturation at 94 °C (5 min), 30 cycles of denaturation at 94 °C (1 min), annealing at 60 °C (1 min and 30 s), extension at 72 °C (1 min), and final extension at 72 °C (5 min); for *hlyA* gene: initial denaturation at 95 °C (2 min), 35 cycles of denaturation at 95 °C (15 s), annealing at 60 °C (30 s), extension at 72 °C (1 min 30 s), and final extension at 72 °C (10 min) [14].

The PCR reagents were prepared for each gene using the NXT Taq PCR Kit Protocol. The reaction mix of 10 μ l contained 5 μ l of NXT Taq PCR Master Mix(2x), 1 μ l of 10X Loading Colour Buffer, 0.25 μ l of each of primers, 1 μ l of DNA template, and 2.5 μ l nuclease free water.

The reaction mixture without DNA was used as negative control for each experiment. The amplicons with Novel Juice (GeneDix, Inc, Germany) was run in 1.5% agarose gel. Gene Ruler 100 bp DNA ladder (SM0241, Thermo Scientific) and Gene Ruler 100 bp plus DNA ladder (SM0321, Thermo Scientific) were used in electrophoresis. The amplicons were visualised using an UV transilluminator (E-Box, Vilber).

The Big Dye Terminator Kit (ThermoFisher, US) was employed for the sequencing of the amplified products using an ExoSAP purification kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) and Sephadex spin-column protocol (Sigma-Aldrich Corp., St. Louis, MO, USA) for cleaning PCR products. After this, the Sanger sequencing method was followed [32]. The Clustal W and Codon Code Aligner were utilised for the alignment of all the samples. The phylogenetic tree for *L. monocytogenes* strains was drawn using the neighbour-joining method [33] with MEGA X software. In the phylogenetic tree, *L. monocytogenes* strains isolated from cows' milk and serotyped isolates registered in GenBank were used as references for *16s rRNA* and *hlyA* genes [14,15].

2.4. Statistical analysis

In this study, statistical analyses were performed using the SPSS 15.0 statistical package program. The p-values were evaluated at the significance level of 0.05. Categorical variables were shown as number/frequency (N) and

percentage (%). The chi-square test was used to compare categorical variables between groups.

3. Results

3.1. Isolation and identification

A total of 16 (n:128, 12.50%) *L. monocytogenes* strains were isolated, including nine (12.16%) from cream cake samples and seven (12.96%) from ice cream samples. *L. monocytogenes* was isolated from all Vidas-positive samples. Also, *L.monocytogenes* grew on all agars from Vidas-positive samples. Vidas-negative samples were also enriched and plated, but *L. monocytogenes* was not detected on any medium.

3.2. Antibiotic susceptibility test results

All the isolates were susceptible to sulfamethoxazole/ trimethoprim, tetracycline, streptomycin, gentamicin, meropenem, and erythromycin. The numbers of isolates which were resistant to sulbactam/ampicillin, Penicillin G, chloramphenicol and ampicillin were 16, 2, 1, and 1, respectively. Furthermore, 3 isolates showed intermediate resistance to amikacin and 2 to ciprofloxacin (Table 1).

3.3. Results of the sequence analysis

The 16S rRNA and *hlyA* genes of all the isolates were successfully amplified with 1500 and 456 bp, respectively (Figure 1, Figure 2). The comparison with the *L. monocytogenes* reference strains as previously described [14,15] showed a sequence similarity of above 99% for these two genes (Figure 3 and Figure 4). The gene sequences of the isolates were recorded in GenBank and their accession numbers were noted (Table 2).

The comparison of the 16s rRNA gene sequences of the *L. monocytogenes* isolates with the GenBank reference serotypes revealed that the isolate 3183 was serotype 1/2c, the isolate 3185 was serotype 1/2a, and the isolate 3192 was serotype 4. The other isolates could not be serotyped from their sequence results (Figure 3).

The comparison of the *hlyA* gene sequences of the *L. monocytogenes* strains with the GenBank reference strains isolated from milk showed that the strains numbered 3183, 3185, 3186, 3187, 3189, 3190, 3192, 3193, 3194, 3197, and 3198 were closely related to the reference strains isolated from milk. Other isolates were similar to the references isolated from milk; however, phylogenetic differences were found (Figure 4).

3.4. Statistical analysis results

There was no statistically significant difference between the number of *L. monocytogenes* positive cream cake and ice cream samples (P = 0.0893 > 0.05).

4. Discussion

This is the first time that *L. monocytogenes* strains from Turkish cream cakes and ice cream have been studied by DNA sequencing.

Milk and milk products and foods made with them are an important source of *Listeria* infection for human beings [31]. In this study, 74 cream cake and 54 ice cream samples offered for direct sale to consumers were examined for the presence of *L. monocytogenes*. A total of 16 (n:128, 12.50%) *L. monocytogenes* strains were isolated, including 9 (12.16%) from cream cakes and 7

Table 1. Antibiogram results of *L. monocytogenes* strains.

Antibiotic discs	<i>Listeria monocytogenes</i> strains (n:16)			Standard zone diameters (according to the references written in materials and methods)		
	S (Susceptible) (%)	I (Intermediate) (%)	R (Resistant) (%)	S (Susceptible)	I (Intermediate)	R (Resistant)
Amikacin (Oxoid, 30µg)	13 (81.25)	3 (18.75)	0 (0)	≥17	15-16	≤14
Ampicillin (Oxoid, 10µg)	15 (93.75)	-	1 (6.25)	16	-	16
Erythromycin (Oxoid, 15µg)	16 (100)	-	0 (0)	25	-	25
Gentamicin (Oxoid, 10µg)	16 (100)	0 (0)	0 (0)	≥15	13-14	≤12
Chloramphenicol (Oxoid, 30µg)	15 (93.75)	-	1 (6.25)	≥21	-	≤20
Meropenem (Oxoid, 10µg)	16 (100)	-	0 (0)	26	-	26
Penicillin G (Oxoid, 10U)	14 (87.5)	-	2 (12.5)	≥29	-	≤28
Ciprofloxacin (Oxoid, 5µg)	14 (87.5)	2 (12.5)	0 (0)	≥21	16-20	≤15
Streptomycin (Oxoid, 10µg)	16 (100)	0 (0)	0 (0)	≥15	12-14	≤11
Sulbactam/ampicillin 1:1 (Oxoid, 20µg)	0 (0)	0 (0)	16 (100)	≥15	12-14	≤11
Tetracycline (Oxoid, 30µg)	16 (100)	0 (0)	0 (0)	≥19	15-18	≤14
Trimethoprim-sulfamethoxazole (Oxoid, 25µg)	16 (100)	-	0 (0)	29	-	29

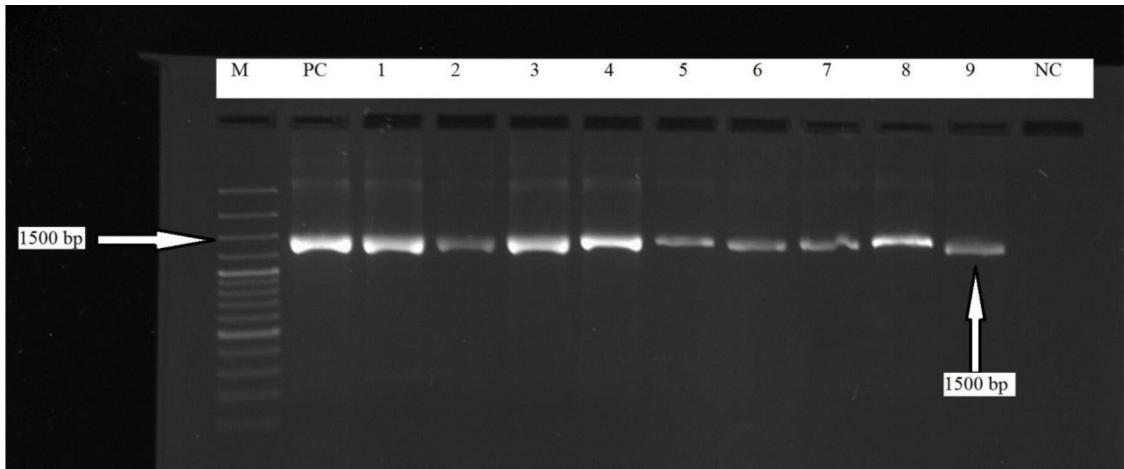


Figure 1. PCR results of *16s rRNA* gene for *L. monocytogenes* strains with 100 bp plus ladder M: Marker, PC: Positive control, NC: Negative control: Line 1-9: positive *L. monocytogenes* strains.

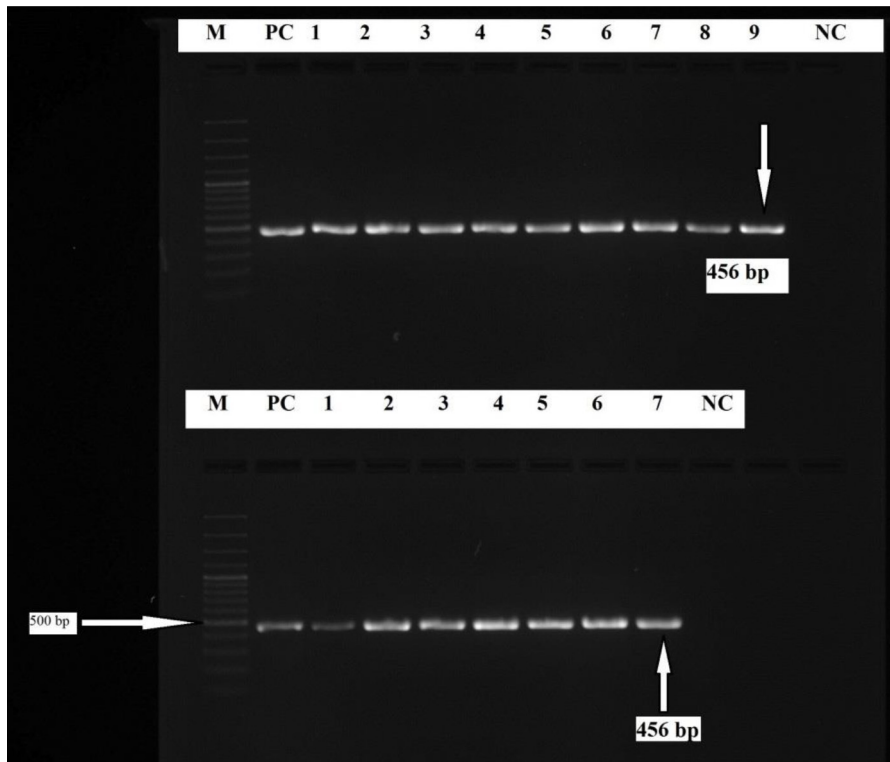


Figure 2. PCR results of *hlyA* gene for *L. monocytogenes* strains. M: Marker, PC: Positive control, NC: Negative control: Line 1-9: positive *L. monocytogenes* strains

(12.96%) from ice cream. Other studies on this topic have been conducted in Turkey in recent years. Aksoy et al. [31] isolated *L. monocytogenes* from 15 of 300 raw milk, butter, and cheese samples. Gönülalan et al. [34] examined 50 ice cream samples and found that five were positive for *L. monocytogenes*. In this study, according to the statistical analysis there is no difference in the number

of *L. monocytogenes* between positive ice cream and cream cakes samples. So, especially all milk products may be a source of infection for *L. monocytogenes* and are thought to be evaluated epidemiologically for this bacterium.

Thaoun et al. [35] isolated 79 strains of *Listeria* spp. from 300 samples, including raw milk, milking equipment, and employees' hand swabs, 69 of which were identified as

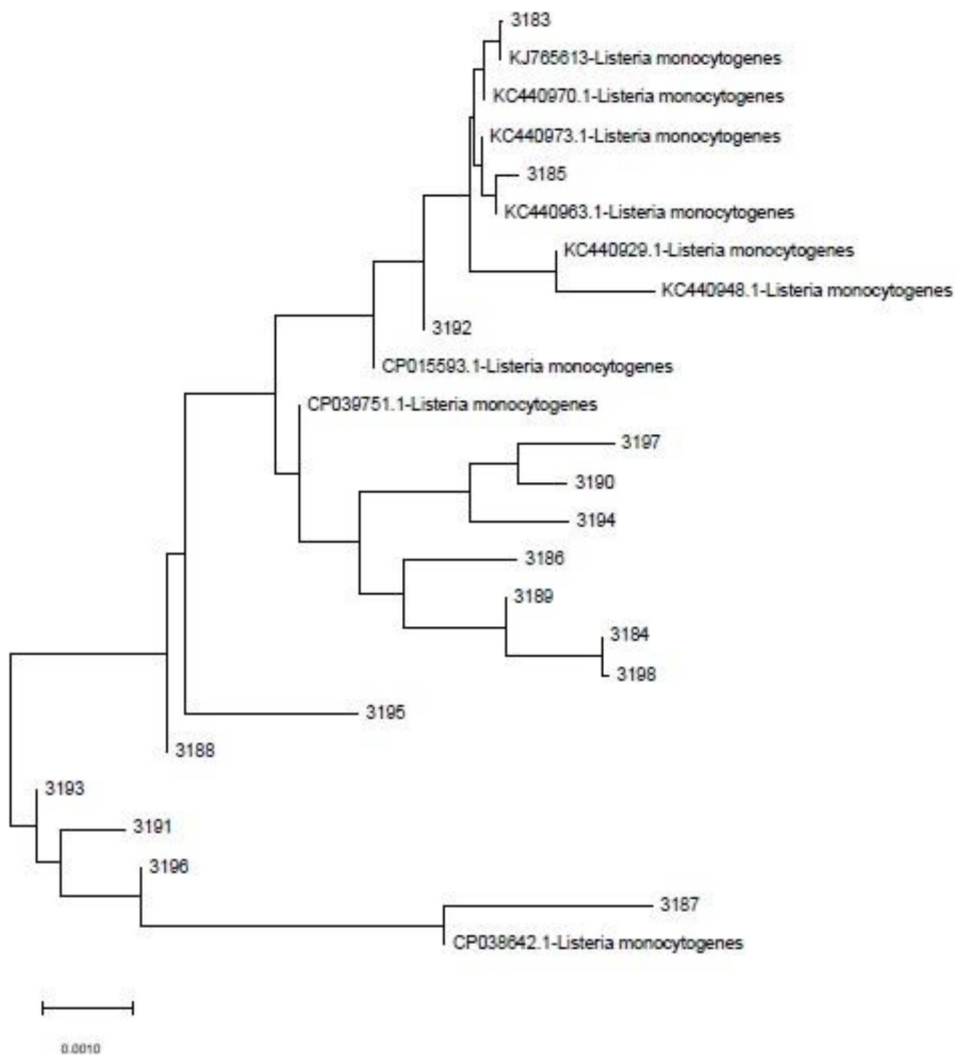


Figure 3. Dendrogram of *16s rRNA* gene for *L. monocytogenes* serotyping.

L. monocytogenes using the Vitek 2 identification system. Akrami-Mohajeri et al.[36] isolated *L. monocytogenes* from 22 (4.03%) of a total of 545 raw milk, butter, cream, and curd cheese samples. Wijendra et al. [37] isolated 80 *L. monocytogenes* from 266 samples including raw milk, pasteurised milk, ice cream, yogurt, cheese, and curd cheese, 10 of which were from ice cream. Mary et al. [38] found *L. monocytogenes* in 32 of 65 ice cream samples. The current results and the studies cited above show that milk and milk *Listeria monocytogenes* is relatively common in milk and milk products. Therefore, *L. monocytogenes* should not be overlooked in husbandry or milk and milk products milk.

In recent years, antibiotic resistance has become of great concern for public health worldwide. The World Health Organisation includes antibiotic resistance within the scope of their One Health concept [39].

The antibiotics to be used especially for people with immunosuppressive diseases such as AIDS patients,

newborn infants, elderly people, and pregnant women, who are the susceptible population, should be limited to *L. monocytogenes*-associated human infections. Nowadays, ampicillin, amoxicillin, erythromycin, tetracycline, trimethoprim/sulfamethoxazole, and imipenem are available for listeriosis treatment. For people allergic to penicillin, trimethoprim/sulfamethoxazole may be used alone or in combination with rifampin [3]. Concerning antibiotic resistance, the resistance genes can be passed between different species, so *L. monocytogenes* could acquire resistance because of antibiotics used against a different pathogen. Antibiotics should not be used as prophylactics or for growth promotion in animals, because this can increase antibiotic resistance, eventually leading to inability to treat infections. In veterinary medicine, the same antibiotics are used in animals and humans for listeriosis, especially in bovine mastitis. Treatment difficulties may arise, because the use of these antibiotics

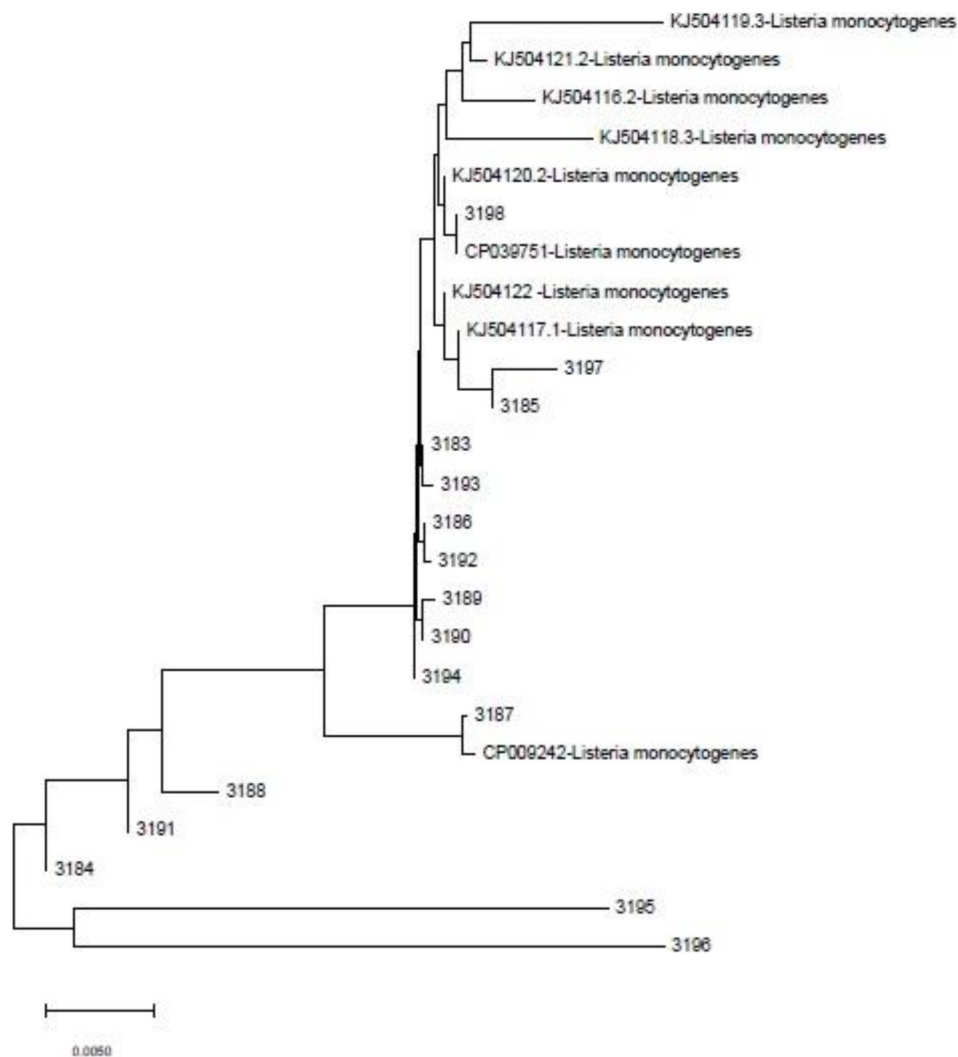


Figure 4. Dendrogram of sequence analysis of *hlyA* gene for *L. monocytogenes* with cows' milk reference strain.

in veterinary medicine can result in the development of resistance in *L. monocytogenes*, which subsequently infects humans. For all these reasons, antibiotic resistance is highly important for both human and animal health within the scope of the One Health concept.

In Turkey, Aksoy et al. [31] stated that among 22 *L. monocytogenes* strains isolated from raw milk and milk products, 1 (6.7%) was resistant to amikacin, meropenem, penicillin G, and vancomycin, and 4 (26.7%) were resistant to trimethoprim/sulfamethoxazole, while all the isolates were susceptible to ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, rifampin, tetracycline, and streptomycin. The results reported in the current study are similar—all the isolates were susceptible to erythromycin, meropenem, streptomycin, tetracycline, gentamicin, and sulfamethoxazole/trimethoprim. These

results show that the antibiotics used for treatment of human listeriosis are currently effective against *L. monocytogenes*. However, a small number of isolates were resistant to penicillin G, chloramphenicol, and ampicillin, so antibiotic resistance should be monitored in the future.

Tahoun et al. [35] stated that of 69 *L. monocytogenes* strains from dairy products in Egypt, 81% were resistant to tetracycline and clindamycin, 71.4% to rifampicin, and 66.7% to gentamicin, and all the isolates were susceptible to ampicillin. Akrami-Mohajeri et al. [36] stated that in Iran, all of 22 *L. monocytogenes* strains isolated from dairy products were susceptible to trimethoprim/sulfamethoxazole, but they were resistant to tetracycline at the rate of 86.3% and to penicillin and chloramphenicol at the rate of 77.2%. The high resistance rates among *L. monocytogenes* isolates especially to tetracycline observed

Table 2. The accession numbers of *L. monocytogenes* isolates deposited in GenBank.

Strain Code	Accession Number	Source of isolate
3183	MN496429	Cream Cake
3184	MN496430	Cream Cake
3185	MN496432	Cream Cake
3186	MN496434	Ice Cream
3187	MN496436	Ice Cream
3188	MN496438	Ice Cream
3189	MN496440	Cream Cake
3190	MN496442	Cream Cake
3191	MN496444	Ice Cream
3192	MN496431	Cream Cake
3193	MN496433	Ice Cream
3194	MN496435	Cream Cake
3195	MN496437	Ice Cream
3196	MN496439	Ice Cream
3197	MN496441	Cream Cake
3198	MN496443	Cream Cake

in these two reports have not been seen in *L. monocytogenes* strains isolated from milk and milk products in Turkey.

Studies carried out to determine the relationship between different bacterial isolates are very important in determining the source of food-borne infections and the irorigin [40,41]. This study shows that the similarities between *L. monocytogenes* strains isolated from cream cakes and ice cream were revealed in the phylogenetic tree constructed using the results of the sequence analysis. However, serotyping of *L. monocytogenes* using only sequence analysis is not adequate. Nucleic acid sequence analyses can be used to determine the similarities between *L. monocytogenes* strains isolated from different foods, the serotypes, and also the sequence analyses of the *hlyA* gene responsible for the haemolytic characteristics of *L. monocytogenes* strains.

References

1. Churchill KJ, Sargeant JM, Farber JM, O'connor AM. Prevalence of *Listeria monocytogenes* in select ready-to-eat foods—deli meat, soft cheese, and packaged salad: A systematic review and meta-analysis. *Journal of Food Protection*. 2019; 82 (2): 344-357. doi:10.4315/0362-028X.JFP-18-158
2. Kocaman N, Sarımehmetoğlu B. Isolation of *Listeria monocytogenes* in lamb meat and determination of the antibiotic resistance. *Ankara University Veterinary Faculty Journal*. 2017; 64 (4): 273-279. doi:10.1501/vetfak_0000002810
3. Procop GW, Church DL, Hall GS, Janda WM, Koneman EW, et al. Koneman's color atlas & textbook of diagnostic microbiology. 7th. ed. Philadelphia: Wolters Kluwer; 2017. pp. 2019-2035
4. Jagadeesan B, Bastic Schmid V, Kupski B, McMahon W, Klijn A. Detection of *Listeria* spp. and *L. monocytogenes* in pooled test portion samples of processed dairy products. *International Journal of Food Microbiology* 2019; 289 (August 2018): 30-39. doi:10.1016/j.ijfoodmicro.2018.08.017

The results of this study show the latest epidemiologic data for *L. monocytogenes* in cream cakes and ice cream in Turkey and also this study is the first attempt to sequence *L. monocytogenes* isolated from cream cakes and ice cream in Turkey. Based on the *16S rRNA* and *hlyA* genes (in BLAST), all the *L. monocytogenes* isolates were similar to the Genbank reference strains isolated from cows' milk. This result showed that ice cream and cream cakes were contaminated by cows' milk used for making cream cakes and ice cream. Cream cakes are stored chilled, and *L. monocytogenes* is able to multiply during their shelf life. Therefore, the inactivation of *L. monocytogenes* should be ensured by pasteurisation of milk (or cream) at sufficient heat and time. Additionally, it is important to prevent contamination of heat-treated milk with raw milk or raw milk-related equipment.

Finally, since *L. monocytogenes* causes mastitis in cows and the infected cows can shed *L. monocytogenes* into their milk, cream cake and ice cream samples could be strongly contaminated from milk used in their production. However, further studies are needed on *L. monocytogenes* to determine its prevalence and antibiotic susceptibility, especially in bovine diseases like mastitis, abortion and encephalitis. At present, *L. monocytogenes* is susceptible to tetracycline, meropenem, trimethoprim/sulfamethoxazole, rifamycin, and gentamicin. However, it is important to monitor resistance in the future, because these antibiotics are used to treat listeriosis in both animals and humans.

Acknowledgments

The author would like to thank Assoc. Prof. Dr. Emre Keskin, an academic member at Ankara University, the Faculty of Aquaculture for his support in sequence analyses, and Ahmet Akçay, the manager of Giresun Food Control Laboratory, the author's former institution, for his support.

Conflict of Interest

The author states no conflict of interest.

5. Lee S, Cappato L, Guimarães J, Balthazar C, Rocha R, et al. *Listeria monocytogenes* in milk: occurrence and recent advances in methods for inactivation. *Beverages* 2019; 5(1): 14. doi:10.3390/beverages5010014
6. Lianou A, Moschonas G, Nychas GJE, Panagou EZ. Growth of *Listeria monocytogenes* in pasteurized vanilla cream pudding as affected by storage temperature and the presence of cinnamon extract. *Food Reserach International* 2018; 106 (November 2017): 1114-1122. doi:10.1016/j.foodres.2017.11.027
7. Buchanan RL, Gorris LGM, Hayman MM, Jackson TC, Whiting RC. A review of *Listeria monocytogenes* : An update on outbreaks, virulence, dose-response, ecology and risk assessments. *Food Control* 2017; 75: 1–13.
8. Singh A, Kour H, Dogra S, Dogra S. Contamination of dairy products by *Listeria monocytogenes* and its control. *Imperial Journal of Interdisciplinary Research* 2017; 1: 1293-1295.
9. Rahimi E, Ameri M, Momtaz H. Prevalence and antimicrobial resistance of *Listeria* species isolated from milk and dairy products in Iran. *Food Control* 2010; 21 (11): 1448-1452. doi:10.1016/j.foodcont.2010.03.014
10. Şanlıbaba P, Uymaz Tezel B, Çakmak GA. Detection of *Listeria* spp. in raw milk and dairy products retailed in Ankara. *Journal of Food* 2018; 43 (2): 273-282. doi:10.15237/gida.gd17107
11. Silbernagel KM, Carver CN, Jechorek RB, Johnson RL. Evaluation of VIDAS *Listeria monocytogenes* II (LMO2) immunoassay method for the detection of *Listeria monocytogenes* in foods: collaborative study. *Food Biological Contaminants* 2004; 87 (5): 1123-1132.
12. Ligozzi M, Bernin C, Bonora MG, de Fatima M, Zuliani J, et al. Evaluation of the VITEK 2 system for identification and antimicrobial susceptibility testing of medically relevant gram-positive cocci. *Journal of Clinical Microbiology* 2002; 40 (5): 1681-1686.
13. Woese CR. Bacterial evolution. *Microbiology Reviews* 1987; 51: 221–227.
14. Soni DK, Dubey SK. Phylogenetic analysis of *Listeria monocytogenes* based on sequencing of 16S rRNA and hlyA genes. *Molecular Biology Reports* 2014; 41 (12): 8219-8229. doi:10.1007/s11033-014-3724-2
15. Hellberg RS, Martin KG, Keys AL, Haney CJ, Shen Y, et al. 16S rRNA partial gene sequencing for the differentiation and molecular subtyping of *Listeria* species. *Food Microbiology* 2013; 36 (2): 231-240. doi:10.1016/j.fm.2013.06.001
16. Moreno LZ, Paixaõ R de Gobbi DDS, Raimundo DC, Ferreira TSP, Moreno AK, et al. Phenotypic and genotypic characterization of atypical *Listeria monocytogenes* and *Listeria innocua* isolated from swine slaughterhouses and meat markets. *Biomedical Research International* 2014; 2014: 742032 doi:10.1155/2014/742032
17. Ricci A, Allende A, Bolton D, Chemaly M, Davies R, et al. *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU. *EFSA Journal*. 2018; 16 (1): 5134. doi:10.2903/j.efsa.2018.5134
18. Hossain MK, Paul S, Hossain MM, Islam MR, Alam MGS. Bovine mastitis and its therapeutic strategy doing antibiotic sensitivity test. *Austin Journal of Veterinary Science & Animal Husbandry* 2017; 4 (1): 1030.
19. Anonymous. Turkish Food Codex Microbiological Criteria Rules. *Official Gazette of the Republic of Turkey* 2011; 28157 (3).
20. Abdollahzadeh E, Ojagh SM, Hosseini H, Irajian G, Ghaemi EA. Prevalence and molecular characterization of *Listeria* spp. and *Listeria monocytogenes* isolated from fish, shrimp, and cooked ready-to-eat (RTE) aquatic products in Iran. *Lwt-Food Science and Technology* 2016; 73: 205-211. doi:10.1016/j.lwt.2016.06.020
21. Arslan S, Özdemir F. Prevalence and antimicrobial resistance of *Listeria* spp. in homemade white cheese. *Food Control* 2008; 19 (4): 360-363. doi: 10.1016/j.foodcont. 2007.04.009
22. Kevenk TO, Terzi Gulel G. Prevalence, antimicrobial resistance and serotype distribution of *Listeria monocytogenes* isolated from raw milk and dairy products. *Journal of Food Safety* 2016; 36: 11-18. doi: 10.1111/jfs.12208
23. Sağun E, Sancak YC, İşleyici O, Ekici K. The presence and prevalence of *Listeria* species in milk and herby cheese in and around Van. *Turkish Journal of Veterinary & Animal Sciences* 2001; 25: 15-19.
24. Erol I, Şireli UT. Occurrence and contamination levels of *Listeria* spp. in milk and dairy products in Ankara. *FEMS Symposium on the versatility of Listeria species* 2002; 10: 11-12.
25. International Standards Institute (ISO). 11290-1. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 1: Detection method. 2017.
26. Johnson R, Mills J. VIDAS® *Listeria monocytogenes* II (LMO2): GovVal validation comparison to the health canada mfhpb-30 reference method for detection of *Listeria monocytogenes* in ready-to-eat meats. *Journal of AOA C International* 2013; 96 (2): 246-250.
27. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* 1966; 45: 493-496.
28. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty second informational supplement (Vol. 32, No. 3). Wayne, PA, USA: Clinical and Laboratory Standards Institute, M100-S22. 2012.
29. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty forth informational supplement (Vol. 34, No. 1). Wayne, PA, USA: Clinical and Laboratory Standards Institute, M100-S24. 2014.
30. European Committee on Antimicrobial Susceptibility Testing (EUCAST). (2019). Breakpoint tables for interpretation of MICs and zone diameters. Version 9.0.

31. Aksoy A, Sezer Ç, Vatansever L, Gülbaz G. Presence and antibiotic resistance of *Listeria monocytogenes* in raw milk and dairy products. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* 2018; 24 (3): 415-421. doi:10.9775/kvfd.2017.19081
32. Sanger F, Coulson AR. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *Journal of Molecular Biology* 1975; 94 (3): 441-448. doi:10.1016/0022-2836(75)90213-2
33. Saitou, N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 1987; 4 (4): 406-425. doi:10.1093/oxfordjournals.molbev.a040454
34. Gönülalan S, Gönülalan Z. Detection of *Listeria monocytogenes* in ice cream samples retailed in Kayseri city of Turkey. *Journal of Health Sciences* 2010; 19 (3): 191-195.
35. Tahoun ABMB, Abou Elez RMM, Abdelfatah EN, Elsohaby I, El-Gedawy AA, et al. *Listeria monocytogenes* in raw milk, milking equipment and dairy workers: molecular characterization and antimicrobial resistance patterns. *Journal of Global Antimicrobial Resistance*. 2017; 10: 264-270. doi:10.1016/j.jgar.2017.07.008
36. Akrami-Mohajeri F, Derakhshan Z, Ferrante M, Hamidiyan N, Soleymani M, et al. The prevalence and antimicrobial resistance of *Listeria* spp in raw milk and traditional dairy products delivered in Yazd, central Iran (2016). *Food and Chemical Toxicology* 2018; 114: 141-144. doi:10.1016/j.fct.2018.02.006
37. Wijendra WAS, Kulathunga KACG, Ramesh R. First report of *Listeria monocytogenes* serotypes detected from milk and milk products in Sri Lanka. *Advances in Animal and Veterinary Sciences* 2014; 2: 11-16.
38. Mary MS, ND S. Pervasiveness of *Listeria monocytogenes* in milk and dairy products. *Journal of Food: Microbiology, Safety & Hygiene* 2017; 02 (03). doi.org/10.4172/2476-2059.1000125
39. Wang W, Arshad MI, Khurshid M, Rasool MH, Nisar MA, Aslam MA, Qamar MU. Antibiotic resistance : a rundown of a global crisis. *Infection and Drug Resistance* 2018; 11: 1645-1658.
40. Bruce JL, Hubner RJ, Cole EM, McDowell CI, Webster JA. Sets of EcoRI fragments containing ribosomal RNA sequences are conserved among different strains of *Listeria monocytogenes*. *Proceedings of the National Academy of Sciences* 1995; 92: 5229-5234
41. Jeffers GT, Bruce JL, McDonough P, Scarlet J, Boor KJ, et al. Comparative genetic characterisation of *Listeria monocytogenes* isolates for human and animal listeriosis cases. *Microbiology* 2001; 147: 1095-1104.